

Claims

1. A method for synthesising one or more bifunctional complexes each comprising
- 5 a) a molecule resulting from the reaction of a plurality of chemical entities and b) an identifier polynucleotide identifying one or more or all of the chemical entities having participated in the synthesis of the molecule, said method comprising the steps of
- 10 i) providing a plurality of building blocks at least some of which comprise one or more chemical entities linked to an identifier oligonucleotide,
- ii) providing one or more connector oligonucleotides capable of hybridising to the identifier oligonucleotides of the building blocks provided in step i),
- 15 iii) hybridising identifier oligonucleotides of the building blocks to the one or more connector oligonucleotides,
- iv) ligating identifier oligonucleotides hybridised to connector oligonucleotide(s), thereby generating an identifier polynucleotide comprising covalently linked identifier oligonucleotides at least some of which are linked to one or more chemical entities,
- 20 v) separating the identifier polynucleotide from the one or more optionally ligated connector oligonucleotide(s),
- 25 vi) reacting the chemical entities linked to the identifier polynucleotide in the absence of hybridisation between identifier oligonucleotides and connector oligonucleotides, and
- 30 vii) obtaining a bifunctional complex comprising a molecule resulting from the reaction of the chemical entities, said molecule being linked to an identifier polynucleotide identifying at least some and preferably all of the chemical entities having participated in the synthesis of the molecule.

2. The method of claim 1, wherein at least one building block identifier oligonucleotide or at least one connector oligonucleotide is attached to a solid support.
- 5 3. The method of claim 1 comprising the steps of
- immobilising at least one building block to a solid support,
- 10 hybridising said immobilized building block oligonucleotide to a first connector oligonucleotide,
- hybridising at least one additional building block oligonucleotide to said first connector oligonucleotide,
- 15 ligating building block oligonucleotides hybridised to the connector oligonucleotide,
- separating the connector oligonucleotide from the ligated building block oligonucleotides,
- 20 reacting one or more chemical entities associated with different, ligated and separated building block oligonucleotides,
- obtaining a first bifunctional complex comprising a first molecule, or first molecule precursor, linked to a first identifier polynucleotide identifying at least
- 25 some of the chemical entities having participated in the synthesis of the molecule, or molecule precursor,
- wherein said first bifunctional complex is optionally immobilised to the solid support.
- 30
4. The method of claim 3, wherein said chemical entities are reacted in a reaction compartment from which the connector oligonucleotide has been removed in a washing and/or separation step prior to the reaction of said chemical entities.
- 35

5. The method of claims 3 or 4 comprising the steps of

providing a second connector oligonucleotide,

5 hybridising said second connector oligonucleotide to the identifier polynucleotide of said first bifunctional complex comprising a first molecule precursor,

hybridising at least one further oligonucleotide of a building block to said second connector oligonucleotide,

10

ligating building block oligonucleotides hybridised to the second connector oligonucleotide, wherein at least one of said building block oligonucleotides are hybridised to the first identifier polynucleotide,

15 separating the second connector oligonucleotide from the ligated building block oligonucleotides, for example by diverting the second connector oligonucleotide to another compartment,

20 reacting the first molecule precursor with the one or more chemical entities associated with the ligated building block oligonucleotide(s),

25 obtaining a second bifunctional complex comprising a second molecule, or second molecule precursor, linked to a second identifier polynucleotide identifying at least some of the chemical entities having participated in the synthesis of the second molecule, or second molecule precursor,

wherein said second bifunctional complex is optionally immobilised to a solid support.

30 6. The method of any of claims 1-5, wherein each step for providing a connector and a building block is repeated for different connector oligonucleotides and different further building blocks.

7. The method of any of claims 1 to 6, wherein said bifunctional complex, or a plurality of such complexes, is released from the solid support to which it is immobilised.
- 5 8. The method of any of claims 1 to 7,
- wherein different bifunctional complexes are generated in different reaction compartments,
- 10 wherein at least some of said different bifunctional complexes are combined in a further reaction compartment comprising a plurality of further connector oligonucleotides,
- wherein at least two of said different bifunctional complexes hybridise to a
- 15 further connector oligonucleotide,
- wherein the molecule precursor part of said complexes react, thereby generating a further molecule in the form of a reaction product,
- 20 wherein the identifier polynucleotides of said bifunctional complexes are optionally covalently linked prior to, during, and/or after, the reaction of the molecule precursors,
- wherein the covalently linked identifier polynucleotides are optionally separated
- 25 from the further connector oligonucleotide prior to or after reaction of said molecule precursors.
9. A method for synthesising a bifunctional complex comprising a molecule resulting from the reaction of a plurality of chemical entities, wherein said
- 30 molecule is linked to an identifier polynucleotide identifying one or more of the chemical entities having participated in the synthesis of the molecule, said method comprising the steps of
- i) providing a plurality of building blocks selected from the group
- 35 consisting of

- 5 d) building blocks comprising an identifier oligonucleotide linked to one or more chemical entities,
- e) building blocks comprising an identifier oligonucleotide linked to one or more reactive groups, and
- 10 f) building blocks comprising an identifier oligonucleotide comprising a spacer or hybridisation region, wherein said building blocks comprising a spacer or hybridisation region are preferably connector oligonucleotides to which building blocks of groups a) and b) can hybridise,
- 15 ii) generating a hybridisation complex comprising at least n building blocks by hybridising the identifier oligonucleotide of one building block to the identifier oligonucleotide of at least one other building block,
- wherein n is an integer of 4 or more
- 20 wherein at least 3 of said at least n building blocks comprise a chemical entity,
- wherein no single identifier oligonucleotide is hybridised to all of the remaining identifier oligonucleotides,
- 25 wherein optionally at least one of said building blocks of group c) is immobilised to a solid support, thereby providing a handle to which an oligonucleotide of at least one building block of groups a) or b) can hybridise,
- 30 iii) covalently linking identifier oligonucleotides of building blocks comprising one or more chemical entities, thereby obtaining at least one identifier polynucleotide comprising covalently linked identifier oligonucleotides each associated with one or more chemical entities,
- 35

iv) optionally separating said identifier polynucleotide obtained in step iii) from any optionally immobilised connector oligonucleotides hybridised thereto, wherein said separation optionally comprises the step of diverting said identifier polynucleotide comprising covalently linked identifier oligonucleotides each associated with one or more chemical entities to a different reaction compartment, thereby separating said identifier polynucleotide from said optionally immobilised connector oligonucleotides

10

v) reacting said at least 3 chemical entities linked to the identifier polynucleotide in the absence of hybridisation between identifier oligonucleotides and connector oligonucleotides, and

15

vi) obtaining a bifunctional complex comprising a molecule resulting from the reaction of a plurality of chemical entities, wherein said molecule is linked to an identifier polynucleotide identifying one or more of the chemical entities having participated in the synthesis of the molecule;

20

preferably, all chemical entities to be reacted are linked to the same identifier nucleotide.

25

10. The method of claim 9 wherein a plurality of different bifunctional complexes is obtained by repeating the method steps for different building blocks.

30

11. The method of any of claims 9 and 10, comprising reacting at least 3 chemical entities, such as at least 4 chemical entities, for example at least 5 chemical entities, such as at least 6 chemical entities.

12. The method of any of claims 1 to 11,

wherein a plurality of molecules are synthesised,

wherein the plurality of synthesised molecules are selected from the group consisting of α -peptides, β -peptides, γ -peptides, ω -peptides, mono-, di- and tri-substituted α -peptides, β -peptides, γ -peptides, ω -peptides, peptides wherein the amino acid residues are in the L-form or in the D-form, vinylogous polypeptides, glycopoly-peptides, polyamides, vinylogous sulfonamide peptides, polysulfonamides, conjugated peptides comprising e.g. prosthetic groups, polyesters, polysaccharides, polycarbamates, polycarbonates, polyureas, polypeptidylphosphonates, polyurethanes, azatides, oligo N-substituted glycines, polyethers, ethoxyformacetal oligomers, poly-thioethers, polyethylene glycols (PEG), polyethylenes, polydisulfides, polyarylene sulfides, polynucleotides, PNAs, LNAs, morpholinos, oligo pyrrolinones, polyoximes, polyimines, polyethyleneimines, polyimides, polyacetals, polyacetates, polystyrenes, polyvinyl, lipids, phospholipids, glycolipids, polycyclic compounds comprising e.g. aliphatic or aromatic cycles, including polyheterocyclic compounds, proteoglycans, and polysiloxanes, including any combination thereof,

wherein each molecule is synthesised by reacting a plurality of chemical entities preferably in the range of from 2 to 200, for example from 2 to 100, such as from 2 to 80, for example from 2 to 60, such as from 2 to 40, for example from 2 to 30, such as from 2 to 20, for example from 2 to 15, such as from 2 to 10, such as from 2 to 8, for example from 2 to 6, such as from 2 to 4, for example 2, such as from 3 to 100, for example from 3 to 80, such as from 3 to 60, such as from 3 to 40, for example from 3 to 30, such as from 3 to 20, such as from 3 to 15, for example from 3 to 15, such as from 3 to 10, such as from 3 to 8, for example from 3 to 6, such as from 3 to 4, for example 3, such as from 4 to 100, for example from 4 to 80, such as from 4 to 60, such as from 4 to 40, for example from 4 to 30, such as from 4 to 20, such as from 4 to 15, for example from 4 to 10, such as from 4 to 8, such as from 4 to 6, for example 4, for example from 5 to 100, such as from 5 to 80, for example from 5 to 60, such as from 5 to 40, for example from 5 to 30, such as from 5 to 20, for example from 5 to 15, such as from 5 to 10, such as from 5 to 8, for example from 5 to 6, for example 5, such as from 6 to 100, for example from 6 to 80, such as from 6 to 60, such as from 6 to 40, for example from 6 to 30, such as from 6 to 20, such as from 6 to 15, for example from 6 to 10, such as from 6 to 8, such as 6, for example from 7 to 100, such as from 7 to 80, for example from 7 to 60, such as from 7 to 40, for

example from 7 to 30, such as from 7 to 20, for example from 7 to 15, such as
from 7 to 10, such as from 7 to 8, for example 7, for example from 8 to 100, such
as from 8 to 80, for example from 8 to 60, such as from 8 to 40, for example
from 8 to 30, such as from 8 to 20, for example from 8 to 15, such as from 8 to
5 10, such as 8, for example 9, for example from 10 to 100, such as from 10 to 80,
for example from 10 to 60, such as from 10 to 40, for example from 10 to 30,
such as from 10 to 20, for example from 10 to 15, such as from 10 to 12, such
as 10, for example from 12 to 100, such as from 12 to 80, for example from 12 to
10 60, such as from 12 to 40, for example from 12 to 30, such as from 12 to 20, for
example from 12 to 15, such as from 14 to 100, such as from 14 to 80, for
example from 14 to 60, such as from 14 to 40, for example from 14 to 30, such
as from 14 to 20, for example from 14 to 16, such as from 16 to 100, such as
from 16 to 80, for example from 16 to 60, such as from 16 to 40, for example
from 16 to 30, such as from 16 to 20, such as from 18 to 100, such as from 18 to
15 80, for example from 18 to 60, such as from 18 to 40, for example from 18 to 30,
such as from 18 to 20, for example from 20 to 100, such as from 20 to 80, for
example from 20 to 60, such as from 20 to 40, for example from 20 to 30, such
as from 20 to 25, for example from 22 to 100, such as from 22 to 80, for example
from 22 to 60, such as from 22 to 40, for example from 22 to 30, such as from 22
20 to 25, for example from 25 to 100, such as from 25 to 80, for example from 25 to
60, such as from 25 to 40, for example from 25 to 30, such as from 30 to 100, for
example from 30 to 80, such as from 30 to 60, for example from 30 to 40, such
as from 30 to 35, for example from 35 to 100, such as from 35 to 80, for example
from 35 to 60, such as from 35 to 40, for example from 40 to 100, such as from
25 40 to 80, for example from 40 to 60, such as from 40 to 50, for example from 40
to 45, such as from 45 to 100, for example from 45 to 80, such as from 45 to 60,
for example from 45 to 50, such as from 50 to 100, for example from 50 to 80,
such as from 50 to 60, for example from 50 to 55, such as from 60 to 100, for
example from 60 to 80, such as from 60 to 70, for example from 70 to 100, such
30 as from 70 to 90, for example from 70 to 80, such as from 80 to 100, for example
from 80 to 90, such as from 90 to 100.

13. The method of any of claims 1 to 12, wherein the molecule is a small molecule
comprising a plurality of functional groups, said small molecule having been
35 generated by reaction of a plurality of chemical entities, wherein said functional

groups are linked by covalent bonds comprising one or more chemical bonds selected from the group consisting of peptide bonds, sulfonamide bonds, ester bonds, saccharide bonds, carbamate bonds, carbonate bonds, urea bonds, phosphonate bonds, urethane bonds, azatide bonds, peptoid bonds, ether bonds, ethoxy bonds, thioether bonds, single carbon bonds, double carbon bonds, triple carbon bonds, disulfide bonds, sulfide bonds, phosphodiester bonds, oxime bonds, imine bonds, imide bonds, including any combination thereof.

- 10 14. The method of any of claims 1 to 12, wherein the molecule is a small molecule comprising a plurality of functional groups, said small molecule having been generated by reaction of a plurality of chemical entities, wherein said functional groups are linked by covalent bonds comprising one or more chemical bonds selected from the group consisting of -NHN(R)CO- ; -NHB(R)CO- ; -
- 15 NHC(RR')CO- ; -NHC(=CHR)CO- ; -NHC₆H₄CO- ; -NHCH₂CHRCO- ; -NHCHRCH₂CO- ; -COCH₂- ; -COS- ; -CONR- ; -COO- ; -CSNH- ; -CH₂NH- ; -CH₂CH₂- ; -CH₂S- ; -CH₂SO- ; -CH₂SO₂- ; -CH(CH₃)S- ; -CH=CH- ; -NHCO- ; -NHCONH- ; -CONHO- ; -C(=CH₂)CH₂- ; -PO₂NH- ; -PO₂CH₂- ; -PO₂CH₂N⁺- ; -SO₂NH- ; and lactams, including any combination thereof.
- 20 15. The method of any of claims 1 to 14, wherein said method results in the synthesis of more than or about 10³ different molecules, such as more than or about 10⁴ different molecules, for example more than or about 10⁵ different molecules, such as more than or about 10⁶ different molecules, for example
- 25 more than or about 10⁷ different molecules, such as more than or about 10⁸ different molecules, for example more than or about 10⁹ different molecules, such as more than or about 10¹⁰ different molecules, for example more than or about 10¹¹ different molecules, such as more than or about 10¹² different molecules, for example more than or about 10¹³ different molecules, such as
- 30 more than or about 10¹⁴ different molecules, for example more than or about 10¹⁵ different molecules, such as more than or about 10¹⁶ different molecules, for example more than or about 10¹⁷ different molecules, such as more than or about 10¹⁸ different molecules.

16. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the molecule does not comprise or consist of an α -peptide.
- 5 17. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the molecule does not comprise or consist of a monosubstituted α -peptide.
- 10 18. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the molecule does not comprise or consist of a nucleotide.
- 15 19. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the identifier polynucleotide is not a natural nucleotide when the molecule is an α -peptide.
- 20 20. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the identifier polynucleotide does not consist exclusively of natural nucleotides, when the molecule is a peptide comprising exclusively monosubstituted α -amino acids.
- 25 21. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the identifier polynucleotide does not consist entirely of natural nucleotides, when the molecule is a natural α -peptide.
- 30 22. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the identifier polynucleotide comprises non-natural nucleotides, when the molecule is a natural α -peptide.
23. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the identifier polynucleotide does not consist of natural nucleotides, when the molecule is a monosubstituted α -peptide.

24. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the identifier polynucleotide does not consist of natural nucleotides, when the molecule is an α -peptide.
- 5 25. The method of any of claims 1 to 14, wherein the molecule comprises covalently linked, functional groups, and wherein the identifier polynucleotide is not a natural nucleotide, when the molecule is a peptide.
- 10 26. The method of any of claims 1 to 14, wherein the molecule is an oligomer or a polymer comprising at least one repetitive sequence of functional groups.
27. The method of any of claims 1 to 14, wherein the molecule comprises at least three functional groups.
- 15 28. The method of any of claims 1 to 14, wherein the molecule comprises a scaffold portion or residue the reactive sites of which reacted with the building block chemical entities during the synthesis of the molecule.
- 20 29. The method of any of claims 1 to 14, wherein the molecule comprises or consists of amino acids selected from the group consisting of α -amino acids, β -amino acids, γ -amino acids, ω -amino acids.
- 25 30. The method of any of claims 1 to 14, wherein the molecule comprises or consists of natural amino acid residues.
31. The method of any of claims 1 to 14, wherein the molecule comprises or consists of α -amino acids.
- 30 32. The method of any of claims 1 to 14, wherein the molecule comprises or consists of monosubstituted α -amino acids.
33. The method of any of claims 1 to 14, wherein the molecule comprises or consists of disubstituted α -amino acids.
- 35

34. The method of any of claims 1 to 14, wherein the molecule comprises or consists of monosubstituted β -amino acids.
- 5 35. The method of any of claims 1 to 14, wherein the molecule comprises or consists of disubstituted β -amino acids.
36. The method of any of claims 1 to 14, wherein the molecule comprises or consists of trisubstituted β -amino acids.
- 10 37. The method of any of claims 1 to 14, wherein the molecule comprises or consists of tetrasubstituted β -amino acids.
38. The molecule of any of claims 1 to 14, wherein the backbone structure of said β -amino acids comprises or consists of a cyclohexane-backbone and/or a
15 cyclopentane-backbone.
39. The method of any of claims 1 to 14, wherein the molecule comprises or consists of γ -amino acids.
- 20 40. The method of any of claims 1 to 14, wherein the molecule comprises or consists of ω -amino acids.
41. The method of any of claims 1 to 14, wherein the molecule comprises or consists of vinylogous amino acids.
- 25 42. The method of any of claims 1 to 14, wherein the molecule comprises or consists of N-substituted glycines.
43. The method of any of claims 1 to 14, the molecule comprises or consists of at
30 least 2 different functional groups, such as at least 3 different functional groups, for example at least 4 different functional groups, such as at least 5 different functional groups, for example at least 6 different functional groups, such as at least 7 different functional groups, for example at least 8 different functional groups, such as at least 9 different functional groups, for example at least 10
35 different functional groups, such as more than 10 different functional groups.

44. The method of any of claims 1 to 14, wherein the hybridisation of a first building block identifier oligonucleotide to a first connector oligonucleotide occurs sequentially or simultaneously with the hybridisation of a second building block identifier oligonucleotide to a second connector oligonucleotide.
45. The method of any of claims 1 to 14, wherein the immobilisation of the at least one building block identifier oligonucleotide or connector oligonucleotide is formed by association of an affinity pair of binding partners associated to the at least one building block identifier nucleotide or connector oligonucleotide and to a solid support, respectively.
46. The method of any of claims 1 to 14, wherein the reaction of chemical entities involve at least two reactive groups of at least one chemical entity.
47. The method of any of claims 1 to 14, wherein each connector oligonucleotide comprises or consists of a sequence of nucleotides.
48. The method of any of claims 1 to 14, wherein each connector oligonucleotide comprises from 3 to 30 nucleotides.
49. The method of any of claims 1 to 14, wherein one or at least two of the identifier polynucleotides further comprises one or more, such as at least two, priming regions or regions capable of self-hybridisation
50. The method of any of claims 1 to 14, wherein one or at least two of the identifier polynucleotides further comprises at least two PCR priming regions for amplification of the template.
51. The method of any of claims 1 to 14, wherein a plurality of building blocks each comprises a building block oligonucleotide covalently linked to at least one chemical entity.

52. The method of any of claims 1 to 14, wherein at least one of said building blocks comprise a chemical entity comprising a scaffold moiety comprising a plurality of reactive groups.
- 5 53. The method of claim 52, wherein said scaffold moiety reactive groups react with one or more chemical entities of a single building block, or with one or more chemical entities of different building blocks.
- 10 54. The method of claims 1 to 14, wherein the chemical entity of at least one building block is transferred to a recipient reactive group of a chemical entity of another building block, such as a chemical entity comprising a scaffold moiety comprising a plurality of reactive groups.
- 15 55. The method of any of claims 1 to 14, wherein at least one of said chemical entities is selectively cleaved from the building block oligonucleotide of the building block to which it was initially attached.
- 20 56. The method of any of claims 1 to 14, wherein at least one chemical entity is simultaneously reacted with a reactive group of a recipient chemical entity and cleaved from the building block identifier oligonucleotide to which the chemical entity is associated.
- 25 57. The method of any of claims 1 to 14, wherein at least one chemical entity forms one member of an affinity pair with another chemical entity.
- 30 58. The method of claim 57, wherein one of the affinity pairs is selected from biotin and dinitrophenol, and any derivative thereof capable of forming an affinity pair with a binding partner capable of forming said affinity pair with biotin and/or dinitrophenol.
59. The method of any of claims 1 to 14, wherein a building block oligonucleotide or a connector oligonucleotide is protected at the 3' end and/or the 5' end by a protection group.

60. The method of claim 59, wherein at least one building block oligonucleotide or connector oligonucleotide is attached to a solid support through the 3' end protection group or the 5' end protection group.
- 5 61. The method of any of claims 59 and 60, wherein the protection group is photo-cleavable and cleaved by exposure to UV light.
62. The method of claim 59, wherein a phosphate group is formed at the 5' end of an building block oligonucleotide following deprotection thereof, thereby
10 converting the building block oligonucleotide to a substrate for an enzyme comprising a ligase activity.
63. The method of any of claims 1 to 14, wherein a plurality of building blocks are provided, and wherein at least a first subset of said plurality of building blocks
15 are provided sequentially and/or the building block identifier nucleotides are sequentially hybridised to one or more connector oligonucleotides, wherein said sequentially provided and/or hybridised building block oligonucleotides are subsequently ligated, and wherein chemical entities of said first subset of sequentially provided building blocks react before a further subset of building
20 blocks are provided and/or hybridised to the same or different connector oligonucleotides as was hybridised to the first subset of building block identifier oligonucleotides.
64. The method of any of claims 1 to 14, wherein all building block identifier
25 oligonucleotides are hybridised to one or more connector oligonucleotides simultaneously or in a single batch reaction.
65. The method of any of claims 1 to 14, wherein all building block identifier
30 oligonucleotides are ligated before any chemical entities are reacted.
66. The method of any of claims 1 to 14, wherein at least some neighbouring building block identifier oligonucleotides are ligated by a chemical ligation reaction, thereby covalently linking said neighbouring building block
35 oligonucleotides.

67. The method of claim 66, wherein building block identifier oligonucleotides linked by chemical ligation are selected from the group consisting of
- 5 first oligonucleotides comprising a 3'-OH group and second oligonucleotides comprising a 5'-phosphor-2-methylimidazole group, which groups are reacted to form a phosphodiester internucleoside linkage,
- 10 first oligonucleotides comprising a phosphoimidazolid group at the 3'-end and a phosphoimidazolid group at the 5'-end, which groups are reacted to form a phosphodisester internucleoside linkage,
- 15 first oligonucleotides comprising a 3'-phosphorothioate group and second oligonucleotides comprising a 5'-iodine group, which groups are reacted to form the internucleoside linkage 3'-O-P(=O)(OH)-S-5', and
- 20 first oligonucleotides comprising a 3'-phosphorothioate group and second oligonucleotides comprising a 5'-tosylate, which groups are reacted to form the internucleoside linkage 3'-O-P(=O)(OH)-S-5'.
- 20 68. The method of any of claims 1 to 14, wherein at least some building block identifier oligonucleotides are ligated to the building block identifier oligonucleotide of a neighbouring building block by a ligase, thereby covalently linking said building block identifier oligonucleotides.
- 25 69. The method of claim 68, wherein the ligase is selected from the group consisting of DNA ligase and RNA ligase.
- 30 70. The method of claim 69, wherein the DNA ligase is selected from the group consisting of Taq DNA ligase, T4 DNA ligase, T7 DNA ligase, and *E. coli* DNA ligase.
- 35 71. The method of any of the preceding claims, wherein the separation of the building block identifier polynucleotides from optionally ligated connector oligonucleotide(s) occurs under conditions resulting in the formation of an at least essentially single stranded identifier polynucleotide.

72. The method of claim 71, wherein the separation occurs by subjecting the hybridised building blocks and connector oligonucleotides to denaturing conditions such as e.g. by performing the separation in a media selected from organic solvents, aprotic solvents, acidic solvents, media comprising denaturants, and alkaline solvents.
73. The method of claim 72, wherein the denaturing conditions are obtained by heating the optionally covalently linked connector oligonucleotides and the ligated building block identifier oligonucleotides hybridised thereto to a temperature above the melting temperature of the duplex portion of the molecule, wherein said heating results in said separation.
74. The method of any of claims 1 to 14, comprising the further step of degrading the optionally ligated connector oligonucleotides before any of the chemical entities linked to the covalently-linked building block identifier oligonucleotides are reacted.
75. The method of claim 74, wherein the optionally ligated connector oligonucleotides comprise or consist of RNA which are degraded by an enzyme selected from RNaseH, RNaseA and RNase 1, by weak alkaline conditions (pH 9-10), or by aqueous $\text{Pb}(\text{Ac})_2$.
76. The method of claim 74, wherein the optionally ligated connector oligonucleotides comprise an internucleoside linker comprising a thiophosphate, wherein the template is treated with aqueous iodine.
77. The method of claim 74, wherein the optionally ligated connector oligonucleotides comprise one or more uracil nucleobases, and wherein the optionally ligated connector oligonucleotides are treated with uracil-glycosylase and subsequently with weak acid to degrade the optionally ligated connector oligonucleotides.
78. The method of any of claims 1 to 77 comprising the further step of separating the identifier polynucleotide from one or more optionally ligated connector oligonucleotides before reacting any chemical entities, subsequently reacting the

chemical entities and generating a bifunctional complex comprising a molecule and an identifier polynucleotide consisting solely of ligated building block oligonucleotides, wherein said identifier polynucleotide identifies one or more or all of the chemical entities having participated in the synthesis of the molecule.

5

79. The method of any of claims 1 to 78, wherein the identifier polynucleotide comprises a first binding partner of an affinity pair, and wherein a second binding partner of the affinity pair is associated with a solid support.

10

80. The method of claim 79, wherein the binding of the binding partners of said affinity pair occurs after the identifier polynucleotide has been separated from the optionally ligated connector oligonucleotides.

15

81. The method of any of claims 1 to 80, wherein at least one chemical entity reaction is an acylation reaction.

20

82. The method of any of claims 1 to 85, wherein at least one chemical entity comprises an amine, and wherein an amide bond is formed when at least one chemical entity is reacted.

25

83. The method of any of the preceding claims comprising the further step of cleaving the molecule from the identifier polynucleotide of the bifunctional complex.

30

84. The method of any of claims 1 to 82, wherein the molecule is associated with the template through a single covalent bond.

85. A method for generating a library of bifunctional complexes comprising a molecule and an identifier polynucleotide capable of identifying the chemical entities having participated in the synthesis of the molecule, or identifying the reaction steps having led to the synthesis of the molecule, said method comprising the steps of

hybridising a plurality of building block identifier oligonucleotides to a plurality of connector oligonucleotides each capable of hybridising to one or more building block oligonucleotides, said building block identifier oligonucleotides being linked to one or more chemical entities,

5

covalently linking said building block oligonucleotides hybridised to one or more connector oligonucleotides, thereby generating a plurality of identifier polynucleotides linked to a plurality of non-reacted chemical entities,

10

separating the identifier polynucleotides from the optionally ligated connector oligonucleotides, preferably by degrading the optionally ligated connector oligonucleotides and/or by performing a washing step wherein the identifier polynucleotides are associated with a solid support capable of being separated from non-bound, optionally ligated connector oligonucleotides.

15

reacting chemical entities linked to each of a plurality of different identifier polynucleotides, and

20

generating a library of bifunctional complexes each comprising a different molecule and an identifier polynucleotide identifying the chemical entities having participated in the synthesis of the molecule,

wherein each of the plurality of molecules are generated by reacting at least 2 chemical entities associated with different building block oligonucleotides.

25

86. The method of claim 85, wherein the different molecules comprise different functional groups.

30

87. The method of any of claims 85 and 86, wherein pools each comprising a plurality of building blocks directed to each connector oligonucleotide are added sequentially.

35

88. The method of any of claims 1 to 87 comprising the further step of subjecting the library of bifunctional complexes to a partitioning procedure, such as an enrichment procedure and/or a selection procedure resulting in the enrichment

and/or selection of bifunctional complexes displaying at least one desirable property.

- 5 89. The method of claim 88, wherein the enrichment procedure and/or selection procedure comprises the step of subjecting the library of bifunctional complexes to a molecular target, and selecting bifunctional complexes binding to said molecular target.
- 10 90. The method of claim 88, wherein the enrichment procedure and/or selection procedure employs an assay generating for each bifunctional complex a result allowing a partitioning of the plurality of bifunctional complexes.
- 15 91. The method of any of claims 88 to 90 comprising the further step of obtaining the identifier polynucleotide part of a bifunctional complex from a plurality of said partitioned bifunctional complexes, and optionally separating the identifier polynucleotide from the molecule of the bifunctional complex.
- 20 92. The method of any of claims 88 to 91 comprising the further step of amplifying in one or more steps said plurality of identifier polynucleotides by a linear amplification method or by an exponential amplification method.
- 25 93. The method of claim 92, wherein said amplification generates a heterogeneous population of duplex molecules each comprising complementary identifier oligonucleotides identifying the chemical entities having participated in the synthesis of the molecule of a bifunctional complex.
- 30 94. The method of any of claims 92 and 93 comprising the further step of converting said amplified identifier polynucleotides into duplex molecules each comprising complementary identifier oligonucleotides identifying the chemical entities having participated in the synthesis of the molecule of a bifunctional complex.
- 35 95. The method of any of claims 92 to 94 comprising the further steps of displacing complementary identifier oligonucleotides, thereby generating a population of heterogeneous identifier oligonucleotides, and reannealing said displaced identifier oligonucleotides under conditions wherein homo-duplexes and hetero-

duplexes are formed, wherein said homo-duplexes comprise identifier oligonucleotides originating from identical bifunctional complexes, and wherein said hetero-duplexes comprise identifier oligonucleotides originating from different bifunctional complexes, such as bifunctional complexes comprising different molecules.

5

96. The method of claim 95, wherein homo-duplexes and hetero-duplexes are separated by one or more chemical or enzymatical separation methods, or by physical separation methods.

10

97. The method of claim 95 comprising the further step of isolating homo-duplexes by removal of hetero-duplexes.

98. The method of claim 97, wherein hetero-duplexes are removed by enzymatic degradation.

15

99. The method of claim 98, wherein the enzyme comprises a nuclease activity.

100. The method of any of the claims 98 and 99, wherein the enzyme is selected from T4 endonuclease VII, T4 endonuclease I, CEL I, nuclease S1, or variants thereof.

20

101. The method of any of claims 98 and 99, wherein the enzyme is thermostable.

25

102. The method of any of the claims 88 to 101, wherein the library comprises 1,000 or more different members, such as 10^5 different members, for example 10^6 different members, such as 10^7 different members, for example 10^8 different members, such as 10^9 different members, for example 10^{10} different members, such as 10^{12} different members.

30

103. The method of any of claims 88 to 102, wherein the molecular target is immobilized on a solid support.

104. The method of claim 103, wherein the target immobilized on the support forms a stable or quasi-stable dispersion.
- 5 105. The method of any of the claims 103 and 104, wherein the molecular target comprises a polypeptide.
106. The method of claim 105, wherein the polypeptide is selected from the group consisting of kinases, proteases, phosphatases.
- 10 107. The method of any of the claims 88 to 105, wherein the molecular target comprises an anti-body.
108. The method of any of the claims 88 to 103, wherein the molecular target comprises a nucleic acid.
- 15 109. The method of claim 108, wherein the nucleic acid comprises a DNA aptamer or an RNA aptamer.
110. The method of any of the claims 105 and 106, wherein the target polypeptide is attached to a nucleic acid having templated the synthesis of the polypeptide.
- 20 111. The method of any of claims 95 to 101, wherein any remaining homoduplexes are amplified prior to decoding the identity of the molecule of a bifunctional complex.
- 25 112. The method of claim 95, wherein the steps of identifier oligonucleotide displacement and reannealing are repeated at least once.
- 30 113. The method of any of claims 95 to 112, wherein the identifier polynucleotide and/or optionally ligated connector oligonucleotides are recovered from the selection procedure and reused for a second or further round of molecule synthesis, or used for hybridisation to the nucleotide part of bifunctional complexes forming an enriched library in the form of a subset of an original library.
- 35

114. The method of any of the preceding claims wherein at least 3 chemical entities are reacted, such as at least 4 chemical entities, for example at least 5 chemical entities, such as at least 6 chemical entities, by reacting at least 1 reactive group of each chemical entity.
115. The method of any of the preceding claims, wherein at least 3 building block oligonucleotides are hybridised to at least 2 connector oligonucleotides, wherein at least 3 of said building block oligonucleotides comprise at least 1 chemical entity, such as a chemical entity comprising at least 1 reactive group, wherein at least 1 of said building block oligonucleotides hybridizes to at least 2 connector oligonucleotides, and wherein at least 3 chemical entities are reacted by reacting at least 1 reactive group of each chemical entity, wherein the reaction of said chemical entities results in the formation of the molecule by reacting the reactive groups of the chemical entities, or by covalently linking at least 3 chemical entities provided by separate building block oligonucleotides.
116. The method of any of the preceding claims, wherein at least 4 chemical entities are reacted, such as at least 5 chemical entities are reacted, for example at least 6 chemical entities are reacted, such as at least 8 chemical entities are reacted, by reacting at least 1 reactive group of each chemical entity.
117. The method of any of the preceding claims comprising the steps of hybridizing at least 4 building block oligonucleotides to at least 2 connector oligonucleotides, wherein at least 4 of said building block oligonucleotides comprise at least 1 chemical entity such as a chemical entity comprising at least 1 reactive group,

wherein at least 1 of said building block oligonucleotides hybridizes to at least 2 connector oligonucleotides,

5 wherein at least 4 chemical entities are reacted by reacting at least 1 reactive group of each chemical entity,

10 wherein the reaction of said chemical entities results in the formation of the molecule by reacting the reactive groups of the chemical entities, or by covalently linking at least 4 chemical entities provided by separate building block oligonucleotides.

118. The method of any of the preceding claims, wherein at least 5 chemical entities are reacted, such as at least 6 chemical entities are reacted, 15 for example at least 8 chemical entities are reacted, such as at least 10 chemical entities are reacted, by reacting at least 1 reactive group of each chemical entity.

119. The method of any of the preceding claims comprising the steps of 20 hybridizing at least 5 building block oligonucleotides to at least 2 connector oligonucleotides,

wherein at least 5 of said building block oligonucleotides comprise at least 1 chemical entities, such as a chemical entity comprising at least 1 reactive group, 25

wherein at least 1 of said building block oligonucleotides hybridizes to at least 2 connector oligonucleotides,

30 wherein at least 5 chemical entities are reacted by reacting at least 1 reactive group of each chemical entity,

wherein the reaction of said chemical entities results in the formation of the molecule by reacting the reactive groups of the chemical entities, or by covalently linking at least 5 chemical entities provided by separate building block 35 oligonucleotides.

120. The method of any of the preceding claims, wherein at least 6 chemical entities are reacted, such as at least 7 chemical entities are reacted, for example at least 8 chemical entities are reacted, such as at least 10 chemical entities are reacted by reacting at least 1 reactive group of each chemical entity.
121. The method of any of the preceding claims wherein reacted chemical entities are linked to the oligonucleotide part of different building blocks.
122. The method of any of the preceding claims, wherein different chemical entities are provided by different building blocks.
123. The method of any of the preceding claims, wherein at least one chemical entity of one building block is reacted with at least one chemical entity of another building block.
124. The method of any of the preceding claims, wherein the molecule comprising reacted chemical entities or covalently linked chemical entities is linked to the polynucleotide part of a building block oligonucleotide.
125. The method of claim 124 comprising the further step of cleaving at least one linker linking the molecule comprising reacted chemical entities or covalently linked chemical entities to the polynucleotide part of a building block oligonucleotide.
126. The method of claim 125, wherein all linkers but 1 linker are cleaved, and wherein the linker not cleaved links the molecule to the polynucleotide part of a building block oligonucleotide.
127. The method of any of the preceding claims, wherein building block oligonucleotides hybridized to connector oligonucleotides are linked by covalent bonds before being separated from optionally ligated connector oligonucleotides hybridised thereto.

128. The method of claim 127 comprising the step of performing a polynucleotide extension reaction resulting in individual building block oligonucleotides being linked together by covalent bonds.

5 129. The method of any of the preceding claims, wherein connector oligonucleotides hybridized to building block oligonucleotides are not linked by covalent bonds before being separated from ligated building block oligonucleotides.

10 130. The method of any of claims 1 to 128, wherein connector oligonucleotides are linked when being separated from ligated building block oligonucleotides, preferably by ligating the connector oligonucleotides, optionally by initially performing a polynucleotide extension reaction resulting in individual connector oligonucleotides being linked together by covalent bonds.

15 131. The method of any of claims 1 to 128 comprising the further steps of
linking the building block identifier oligonucleotides, preferably by ligating the
building block identifier oligonucleotides, optionally preceded by performing a
20 polynucleotide extension reaction resulting in individual building block identifier
oligonucleotides being linked together by covalent bonds, and
linking the connector oligonucleotides, preferably by ligating the connector
oligonucleotides, optionally preceded by performing a polynucleotide extension
25 reaction resulting in individual connector oligonucleotides being linked together
by covalent bonds,

said linking reactions being performed prior to the separation of linked building
block identifier oligonucleotides from the linked connector oligonucleotides.

30 132. The method of any of the preceding claims, wherein the method does not involve ribosome mediated translation.

35 133. The method of any of claims 114 and 115 comprising the further step of hybridizing at least 1 further connector oligonucleotide to at least 1 building

block oligonucleotide, such as 2 or more building block oligonucleotides, hybridized to at least 1 connector oligonucleotide, such as 2 or more connector oligonucleotides, of a hybridisation complex comprising hybridised connector oligonucleotides and building block oligonucleotides.

5

134. The method of claim 133, wherein the further connector oligonucleotide is selected from the group consisting of

10

connector oligonucleotides comprising at least 1 chemical entity comprising at least 1 reactive group,

connector oligonucleotides comprising at least 1 reactive group, and

connector oligonucleotides comprising at least 1 spacer region.

15

135. The method of any of claims 114, 115, 133 and 134 comprising the further step of hybridizing at least 1 further building block oligonucleotide selected from the group consisting of

20

building block oligonucleotides comprising at least 1 chemical entity comprising at least 1 reactive group,

building block oligonucleotides comprising at least 1 reactive group, and

25

building block oligonucleotides comprising at least 1 spacer region,

30

to at least 1 connector oligonucleotide, such as 2 connector oligonucleotides of a hybridisation complex, or to the at least 1 further connector oligonucleotide hybridised in the method of claims 133 and 134, wherein said connector oligonucleotide or further connector oligonucleotide is preferably hybridized to at least 1 building block oligonucleotide, such as 2 or more complementary connector oligonucleotides, for example 3 complementary connector oligonucleotides, such as 4 complementary connector oligonucleotides, for example 5 complementary connector oligonucleotides, such as 6

35

complementary connector oligonucleotides.

136. The method of claim 134, wherein the step of hybridizing at least 1 further connector oligonucleotide is repeated at least once, such as 2 times, for example 3 times, such as 4 times, for example 5 times, such as 6 times.
- 5 137. The method of claim 135, wherein the step of hybridising at least one further building block oligonucleotide is repeated at least once, such as 2 times, for example 3 times, such as 4 times, for example 5 times, such as 6 times.
- 10 138. The method of any of the preceding claims, wherein at least n connector oligonucleotides and at least $n-1$ building block oligonucleotides are provided, n being an integer of from 3 to 6, and wherein each building block oligonucleotide hybridizes to at least 2 connector oligonucleotides.
- 15 139. The method of claim 138, wherein n is 3 or 4.
140. The method of any of claims 1 to 137, wherein at least n connector oligonucleotides and at least n building block oligonucleotides are provided, n being an integer of from 3 to 6, and wherein at least $n-1$ building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
- 20 141. The method of claim 140, wherein n building block oligonucleotides hybridize to at least 2 connector oligonucleotides.
- 25 142. The method of any of claims 140 and 141, wherein n is 3 or 4.
143. The method of any of claims 1 to 137, wherein at least n connector oligonucleotides and at least $n+1$ building block oligonucleotides are provided, n being an integer of from 3 to 6, and wherein at least $n-1$ building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
- 30 144. The method of claim 143, wherein n building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
- 35 145. The method of any of claims 143 and 144, wherein n is 3 or 4.

146. The method of any of claims 1 to 137, wherein at least n connector oligonucleotides and at least $n+2$ building block oligonucleotides are provided, n being an integer of from 3 to 6, and wherein at least $n-1$ building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
147. The method of claim 146, wherein n building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
148. The method of any of claims 146 and 147, wherein n is 3 or 4.
149. The method of any of claims 1 to 137, wherein at least n connector oligonucleotides and at least $n+3$ building block oligonucleotides are provided, n being an integer of from 3 to 6, and wherein at least $n-1$ building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
150. The method of claim 149, wherein n building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
151. The method of any of claims 149 and 150, wherein n is 3 or 4.
152. The method of any of claims 1 to 137, wherein at least n connector oligonucleotides and at least $n+4$ building block oligonucleotides are provided, n being an integer of from 3 to 6, and wherein at least $n-1$ building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
153. The method of claim 152, wherein n building block oligonucleotide hybridize to at least 2 connector oligonucleotides.
154. The method of any of claims 152 and 153, wherein n is 3 or 4.
155. The method of any of claims 1 to 137, wherein said plurality of connector oligonucleotides comprises branched connector oligonucleotides, wherein at least n branched connector oligonucleotides and at least n building block oligonucleotides are provided, n being an integer of from 2 to 6, and

wherein at least $n-1$ building block oligonucleotide hybridize to at least 2 branched connector oligonucleotides.

5 156. The method of claim 155, wherein at least $n+1$ building block oligonucleotides are provided.

10 157. The method of any of claims 155 and 156, wherein at least n building block oligonucleotides hybridize to at least 2 branched connector oligonucleotides.

158. The method of claim 157, wherein at least $n+1$ building block oligonucleotide hybridize to at least 2 connector oligonucleotides.

15 159. The method of any of claims 157 to 158, wherein n is 3 or 4.

20 160. The method of any of the preceding claims comprising the further step of repeating at least once, such as 2 times, for example 3 times, such as 4 times, for example 5 times, such as 6 times, for different connector oligonucleotides and different building block oligonucleotides, the steps of hybridising connector oligonucleotides and building block oligonucleotides to a hybridisation complex comprising hybridised connector oligonucleotides and building block oligonucleotides.

25 161. The method of any of claims 1 to 160, wherein a plurality of reactive groups of at least 1 chemical entity of a building block oligonucleotide react with reactive groups of chemical entities of at least 2 other building block oligonucleotides.

30 162. The method of claim 161, wherein the at least 1 chemical entity comprises from 2 to 6 reactive groups.

35 163. The method of claim 161, wherein at least 3 of said reactive groups of said at least 1 chemical entity react with at least 1 reactive group of at least 3 additional chemical entities.

164. The method of any of the preceding claims, wherein said plurality of building block oligonucleotides comprise at least 2 building block oligonucleotides which are non-identical.
- 5 165. The method of any of the preceding claims, wherein said plurality of building block oligonucleotides comprise at least 2 branched building block oligonucleotides.
- 10 166. The method of any of the preceding claims, wherein said plurality of connector oligonucleotides comprise connector oligonucleotides comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 100, such as less than 80, for example less than 60, such as less than 40.
- 15 167. The method of claim 166, wherein said plurality of connector oligonucleotides further comprise connector oligonucleotides comprising at least 1 branching point connecting at least three polynucleotide fragments comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 100, such as less than 80, for example less than 60, such as less than 40.
- 20 168. The method of any of claims 1 to 167, wherein said plurality of building block oligonucleotides comprise oligonucleotides comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 60, such as less than 40, for example less than 20.
- 25 169. The method of claim 168, wherein said plurality of building block oligonucleotides further comprise polynucleotides comprising at least 1 branching point connecting at least three polynucleotide fragments comprising a sequence of n nucleotides, wherein n is an integer of from 8 to preferably less than 60, such as less than 40, for example less than 20.
- 30 170. The method of any of the preceding claims, wherein the polynucleotide part of at least one connector oligonucleotide and/or at least one building block oligonucleotide is capable of undergoing self-hybridization.

171. The method of any of the preceding claims comprising the further step of covalently linking at least one connector oligonucleotide to at least one building block oligonucleotide.
- 5 172. The method of any of the preceding claims, wherein the connector oligonucleotides and/or the building block oligonucleotides are provided in batch.
- 10 173. The method of any of claims 1 to 172, wherein the connector oligonucleotides and/or the building block oligonucleotides are provided sequentially.
- 15 174. A method for synthesising a plurality of different molecules, said method comprising
- providing a plurality of connector oligonucleotides each capable of hybridizing to at least 1 complementary connector oligonucleotide,
- providing a plurality of complementary connector oligonucleotides selected from the group consisting of
- 20 d) complementary connector oligonucleotides comprising at least 1 chemical entity comprising at least 1 reactive group,
- e) complementary connector oligonucleotides comprising at least 1 reactive
- 25 group,
- f) complementary connector oligonucleotides comprising at least 1 spacer region,
- 30 hybridizing the plurality of connector oligonucleotides and complementary connector oligonucleotides, thereby forming a plurality of different hybridisation complexes, each hybridisation complex comprising at least 2 complementary connector oligonucleotides and at least 2 connector oligonucleotides,
- 35 wherein, for each of said hybridisation complexes,

at least 2 of said complementary connector oligonucleotides comprise at least 1 chemical entity comprising at least 1 reactive group, and

5 at least 1 of said complementary connector oligonucleotides hybridizes to at least 2 connector oligonucleotides, and

10 ligating, enzymatically, chemically, or otherwise, complementary connector oligonucleotides, thereby forming identifier polynucleotides, wherein each identifier polynucleotide is associated with a plurality of unreacted chemical entities,

15 separating each identifier polynucleotide associated with unreacted chemical entities from optionally ligated connector oligonucleotides associated therewith,

20 reacting, when the identifier polynucleotides are no longer hybridised to the optionally ligated connector oligonucleotides, at least 2 chemical entity reactive groups of each polynucleotide identifier by reacting at least 1 reactive group of each chemical entity,

25 wherein, for each bifunctional complex, the reaction of said chemical entity reactive groups results in the formation of a different molecule by reacting at least 2 chemical entities provided by separate complementary connector oligonucleotides, thereby synthesising a plurality of different molecules.

175. The method of claim 174 comprising the further step of selecting molecules having desirable characteristics, wherein the selection employs a predetermined assaying procedure.

30 176. The method of any of claims 174 to 175 comprising the further step of amplifying at least part of the individual and optionally ligated connector oligonucleotides used for the formation of the initial hybridisation complexes.

177. The method of claim 176 comprising the further step of contacting a population of said amplified, optionally ligated connector oligonucleotides, or fragments thereof, with a plurality of building block oligonucleotides.

5 178. The method of claim 177 comprising the further step of performing an additional synthesis round by carrying out the steps of the method of the invention using a population of said amplified connector oligonucleotides, or a population of said amplified connector oligonucleotide fragments.

10

15

20

25